Effect of Meloidogyne incognita on Selected Forest Tree Species[1]

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Abstract: Four or five growth stages of 14 forest tree species were tested for susceptibility to *Meloidogyne incognita* at five inoculum levels. Responses ranged from the highly susceptible 'China fir' to immune 'Taiwania'. Even highly susceptible species became increasingly tolerant at later growth stages, thus root-knot appears to be a greater problem in nurseries than in established forests. Heavily suberized cells which restricted nematode development was the predominant host response in Norway spruce, and in the jack, scotch, and Virginia pines. Adult females in jack and scotch pine, which elicited a minimum of suberized tissue, were found adjacent to infection sites showing maximum suberization which indicates that resistance can be highly localized and variable within an individual host. A few gravid females, but no giant cells, were observed in these two species. *Key words:* resistance, conifers, hardwoods, pathogenicity, root-knot nematode.

Since the demand for tree seedlings used in Taiwan for reforestation has become increasingly urgent, the continuous and intensive monoculture in nurseries of certain tree species has increased the severity of nematode diseases. In the USA, root-knot nematodes were reported associated with red maple, yellow poplar, American sycamore, river birch, and eastern cottonwood (14). In Japan, root-knot nematodes were reported in 25 of 40 forest nurseries surveyed (7). The larvae of *Meloidogyne incognita* (Kofoid and White) Chitwood, were reported to enter the roots of Virginia pine, pitch pine, and eastern white pine (13), and a *Meloidogyne* sp. was found on short-leaf and slash pines (12). Recently, an undescribed species of *Meloidogyne* was reported to penetrate the mycorrhizal mantle and colonize roots of mature ponderosa pine, and possibly reduce the role of ectomycorrhizae as a biological deterrent to root infection by other pathogens (11). In Taiwan, *Meloidogyne* spp. severely reduced production of *Paulownia fortunei* in a forest nursery when rooted cuttings were planted for two succesive seasons in the same beds (17).

Other forest trees may also be damaged by root-knot nematodes. *M. incognita* has a world-wide distribution in temperate and tropical zones, has a wide host range, and causes serious economic loss to many plant species. In the present study, we tested 14 different forest tree species native both to North America and Taiwan to determine: (i) susceptibility to *M. incognita,* (ii) influence of *M. incognita* on plant growth at different inoculum levels and at different seedling stages, and (iii) host-parasite interactions by histopathological studies.

MATERIALS AND METHODS

The following 14 forest tree species were tested for susceptibility to *M. incognita:* white pine, *Pinus strobus* L.; northern white cedar, *Thuja occidentalis* L.; Norway spruce, *Picea abies* (L.) Karst.; scotch pine, *Pinus sylvestris* L.; jack pine, *Pinus banksiana* Lan.; Virginia pine, *Pinus virginiana* Mill.; red pine, *Pinus resinosa* Sol.; China-fir, *Cunninghamia lanceolata* (Lamb) Hook.; Japanese-fir, *Cryptomeria japonica* D. Don.; yellow cypress, *Chamaecyparis obtusa* Sieb. and Zucc. var. *formosana* (Hay) Rehd.; taiwania, *Taiwania cryptomerioides* Hay.; black locust, *Robinia pseudoacacia* L.; fortune paulownia, *Paulownia fortunei* Hemsl.; and albizzia, *Albizzia falcata* Backer.

Nematode inoculum consisted of newly emerged larvae extracted in a mist chamber from galled tomato roots *Lycopersicon esculentum* Mill. 'Bonny Best'. Larval suspension of predetermined concentrations were prepared and test plants were inoculated in the following growth stages: pre-emergence, cotyledon, two-leaf or two-eight needle, multipetiole, and 1-year-old seedling stages. Inoculum levels were 0, 100, 1,000, and 10,000 larvae per pot except the 1-year-old seedling stage which received an additional level of 20,000. Each treatment was replicated

Received for publication 17 April 1974.

[1]Portion of a M.S. Thesis submitted by the senior author to Purdue University, West Lafayette, Indiana 47907. Journal Series Paper No. 5466, Purdue University Agricultural Experiment Station, West Lafayette, Indiana 47907.

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five times. All seeds were treated with a fungicide (Captan 75%), germinated in a moist chamber at 24 C, and transplanted to 11.5-cm diam clay pots filled with steam-sterilized Chelsea loamy fine sand. In the pre-emergence experiment, the inoculum was placed directly on the germinated seeds before covering them with soil. A nematode-free supernatant solution obtained from the larval suspension was applied to the controls as a check against the introduction of other pathogens along with the larval inoculum. Seedlings that emerged were counted, and, 40 days after inoculation, surviving seedlings were counted. In the cotyledon, two-leaf or two-eight needle, and multipetiole experiments, seedlings were thinned to one to three seedlings per pot, and the inoculum was poured into a 4-cm deep trench 3 cm from the plant. One-year-old seedlings, transplanted from methyl bromide-treated nursery beds, were washed thoroughly and singly transplanted to 15-cm diam clay pots filled with steam-sterilized Chelsea loamy fine sand. Seedlings of uniform size were inoculated by pouring nematode larvae into three, 5-cm deep holes around the base of the plants.

All experiments were conducted in a greenhouse at a soil temperature of 24-32 C. Growth of plants was maintained by application weekly of 50 ml per pot of Hoagland's solution (16). Nematode reproduction was determined 8 months after inoculation for conifers, and 6 months after inoculation for hardwoods, by placing the washed root systems in a mist chamber for 1 wk to recover emerged larvae and by observation of eggs and larvae in histological sections. The roots were oven-dried and weighed, and height and dry top weights of seedlings were also recorded. An analysis of variance was computed for each of the experiments.

For histopathological studies, root galls from infected black locust, albizzia, China-fir, scotch pine, jack pine, Norway spruce and Virginia pine seedlings were fixed in an aqueous solution of 10% formalin, 50% ethyl alcohol, and 5% acetic acid (FAA), dehydrated in a tertiary butyl alcohol series and embedded in Tissueprep® (melting point, 61 C). Root galls were sectioned transversely and longitudinally (20 μm) and stained with safranin and fast green (15).

RESULTS

Five categories were used to classify the host's response to infection: (i) Tolerant – plants grew well, had undamaged vigorous root systems and had no effect on infection, reproduction, and development of the nematode. (ii) Susceptible – plants grew poorly, but had no effect on the nematode. (iii) Resistant – plants grew well, but had a hypersensitive or resistant reaction which restricted the infection, reproduction, and development of the nematodes. (iv) Intolerant – plants grew poorly and also limited the infection, reproduction, and development of the nematode (2). Restricted infection did not preclude penetration by the larvae. (v) Immune – no visible evidence of infection.

China-fir: This species was highly susceptible in the pre-emergence stage. All seedlings were killed in soil infested with 10,000 larvae/pot. The number of emerging seedlings was significantly decreased ($P = 0.01$) by 1,000 larvae/pot. Seedling mortality continued after emergence due to severe nematode infection and damping-off. Damping-off was rare in control plants. The number of surviving seedlings 40 days after inoculation was significantly decreased by as few as 100 larvae/pot (Table 1). *Fusarium oxysporum* (Schlecht.) Snyd. and Hans. was isolated consistently from diseased seedlings.

When inoculations were made in the cotyledon stage, highly significant decreases in height and dry top weight were caused by 100, 1,000, and 10,000 larvae/pot (Table 2, Fig. 1-A). When inoculated in the two-eight needle stage, height and dry top weight were significantly ($P = 0.01$) reduced by 1,000 and 10,000 larvae/pot, but not by 100 larvae/pot. When inoculated in the multipetiole stage, growth was significantly ($P = 0.01$) reduced by 10,000 larvae/pot and by 1,000 larvae/pot ($P = 0.5$), but not by 100 larvae/pot. This species was not inoculated in the 1-year-old seedling stage.

M. incognita completed its life cycle on China-fir, but results were highly variable. Recovery of larvae from galled roots in various replications ranged from 0-140; 0-6,308; 48-7,160; and 0-6,314, larvae per root system in pre-emergence, cotyledon, two-eight needle, and multipetiole stages, respectively.

—1) Histopathology in China-fir—China-fir was the only susceptible and partly

FIG. 1-(A to H). Effect of *Meloidogyne incognita* on growth of tree seedlings and histological reaction of host to infection. **A.** China-fir seedlings 6 months after inoculation with *M. incognita.* From left to right: soil infested with 0, 100, 1,000 and 10,000 nematode larvae/pot, respectively. **B.** Longitudinal section showing an adult female nematode (NM) near root tip (RT) and hyperplasia (HA) and hypertrophy (HY) of cells. The galled root tip was increased about three times in size. **C.** Transverse section shows an adult female nematode (NM), giant cells (GC) and egg mass (EM). **D.** A coiled adult male nematode (NM) in the vascular tissue of a longitudinal section. **E.** The effect of *M. incognita* on damping-off of scotch pine after emergence of seedlings. **F.** A typical resistant reaction in scotch pine shows several layers of suberized tissue (BT) around a nematode larvae (NM). **G.** Suberized tissue (BT) induced by the nematode (NM) formed adjacent to compacted tracheids. Hypertrophied and hyperplastic (HA) cells are prevalent in both the cortex and stele (ST). **H.** A normal mature female nematode (NM) adjacent to suberized tissue caused by a resistant reaction to another nematode. Egg mass (EM) on the root surface.

TABLE 1. Effect of *Meloidogyne incognita* on seedling survival 40 days after inoculation.

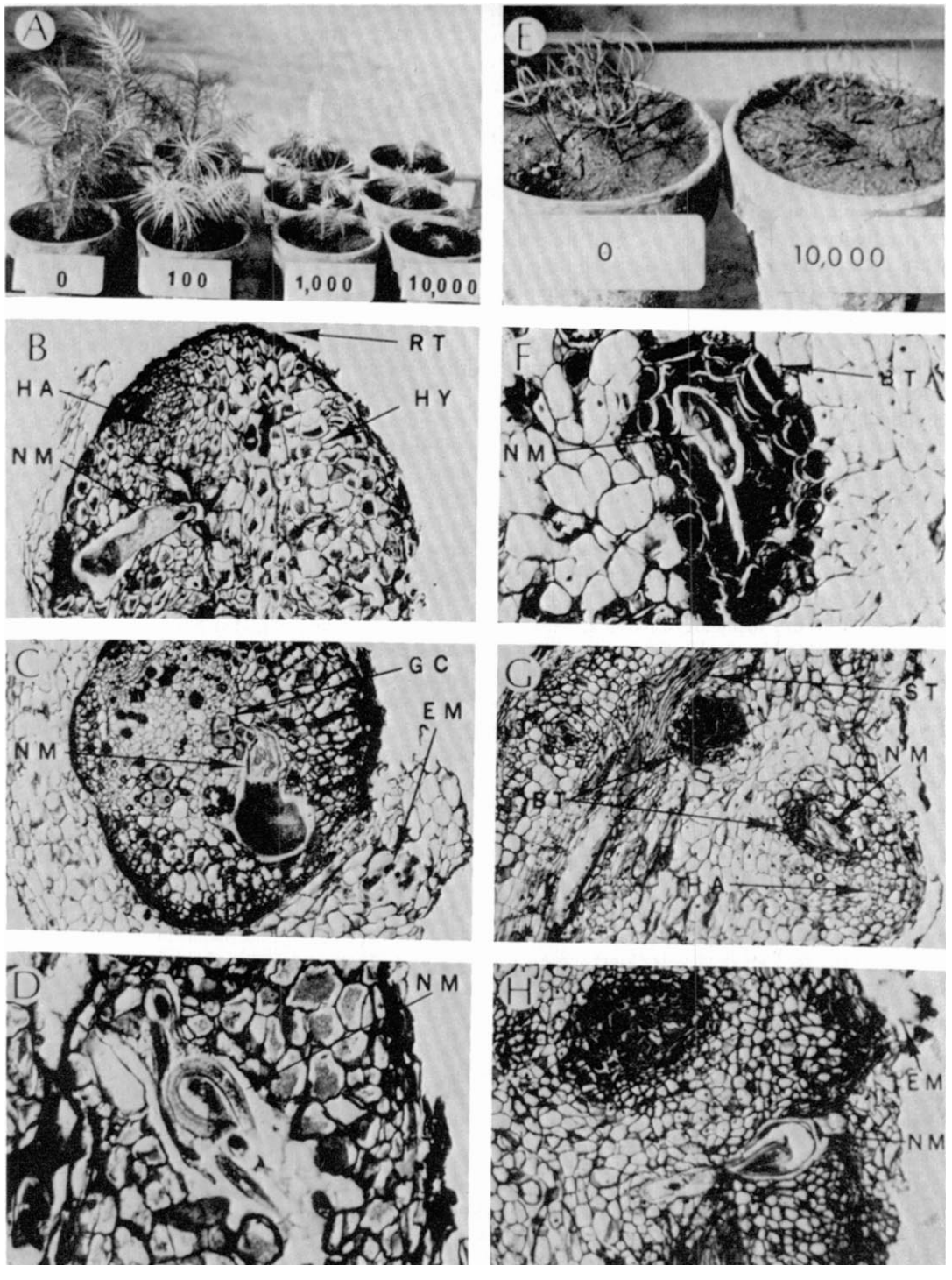
Inoculum[a] (larvae/pot)	Trees tested (No. of surviving seedlings[b]) Black locust	China-fir	Japanese-fir	Yellow cypress	Scotch pine	Virginia pine	Jack pine
10,000	3.00**[c]	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
1,000	10.00	0.40**	0.60*[d]	0.00**	1.80**	1.00**	0.60**
100	9.60	0.60**	0.20**	1.20	4.20*	5.00	6.20**
0	10.00	4.80	1.40	2.00	9.00	5.80	14.60

[a]Applied at planting.
[b]Mean of five replications.
[c]** = Significantly different $P = 0.01$.
[d]* = Significantly different $P = 0.05$.

TABLE 2. Effect of *Meloidogyne incognita* on growth of hardwood and conifer seedlings inoculated in the cotyledon, two-leaf or two-eight needle, and multipetiole stages[a].

Trees tested	Inoculum (larvae/pot)	Cotyledon stage Height (cm)	Cotyledon stage Dry top wt (g)	Two-leaf or two-eight needle stage Height (cm)	Two-leaf or two-eight needle stage Dry top wt (g)	Multipetiole stage Height (cm)	Multipetiole stage Dry top wt (g)
China-fir	10,000	7.67**[b,d]	0.47**	5.84**	0.17**	12.00**	1.98**
	1,000	5.43**	0.38**	4.42**	0.17**	14.35*[c]	2.33*
	100	9.45**	0.81**	8.52	0.88	19.05	2.62
	0	20.66	2.19	16.14	1.37	21.55	4.62
Yellow cypress	10,000	1.80**[b]	0.02**	3.38	0.04	7.16	0.14
	1,000	5.50**	0.05*[c]	6.49	0.10	9.44	0.16
	100	9.70	0.17	9.73	0.19	13.04**	0.35
	0	8.90	0.13	8.00	0.15	8.18	0.12
Fortune paulownia	10,000	17.20**[c]	1.20**	55.40	7.40*	45.80	10.54
	1,000	73.20	10.64	62.20	9.79	45.80	12.21
	100	71.60	11.93	49.00	8.49	55.20**	11.01
	0	66.40	10.47	60.20	9.96	45.20	12.22
Scotch pine	10,000	4.12[b]	0.34	5.58	0.39	5.80	0.48
	1,000	3.54**	0.28	4.92	0.62	6.83	0.50
	100	4.46	0.38	6.88	0.58	8.02	0.75
	0	4.99	0.36	6.99	0.35	6.87	0.53

[a]Mean of five replications.
[b]Determined 8 months after inoculation
[c]Determined 6 months after inoculation.
[d]** = Significantly different, $P = 0.01$.
[e]* = Significantly different, $P = 0.05$.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intolerant (3) species of the 11 conifers tested. Nematodes developed well, but were smaller in size than those in black locust and albizzia, and were found in abundance just behind the root tip (Fig. 1-B). An adult female nematode is shown in Fig. 1-C with the head in contact with giant cells in the primary vascular tissue. Egg masses, but with fewer eggs, were deposited in the cortex or on the root surface through a rupture in the cortex. Mature males were often found coiled in the host vascular tissue, but no giant cells were associated with them (Fig. 1-D).

Multinucleate giant cells had unevenly thickened cell walls, with fewer, but larger nuclei than observed in other hosts (Fig. 1-C). Evidence of limited cell necrosis was observed around the head of the nematodes. Hyperplastic and hypertrophied cells were prevalent in the stele and cortex.

Yellow cypress: All seedlings were killed in soil infested with 1,000 and 10,000 larvae/pot in the pre-emergence stage (Table 1). When inoculated in the cotyledon stage (Table 2), the height and dry top weight was significantly reduced ($P = 0.01$) by 10,000 larvae/pot; at 1,000 larvae/pot the reduction in the height was significant ($P = 0.01$) and the reduction in dry top weight was significant $P = 0.05$. There was no significant reduction in height or dry top weight of seedlings at any inoculum level when inoculated in the two-eight needle and multipetiole stages; however, seedling height was significantly ($P = 0.01$) increased when inoculated with 100 larvae/pot in the multipetiole stage. No inoculations were made in the 1-year-old stage, and no histopathological studies were performed on yellow cypress. Larvae could not be recovered from galled roots in any growth stage.

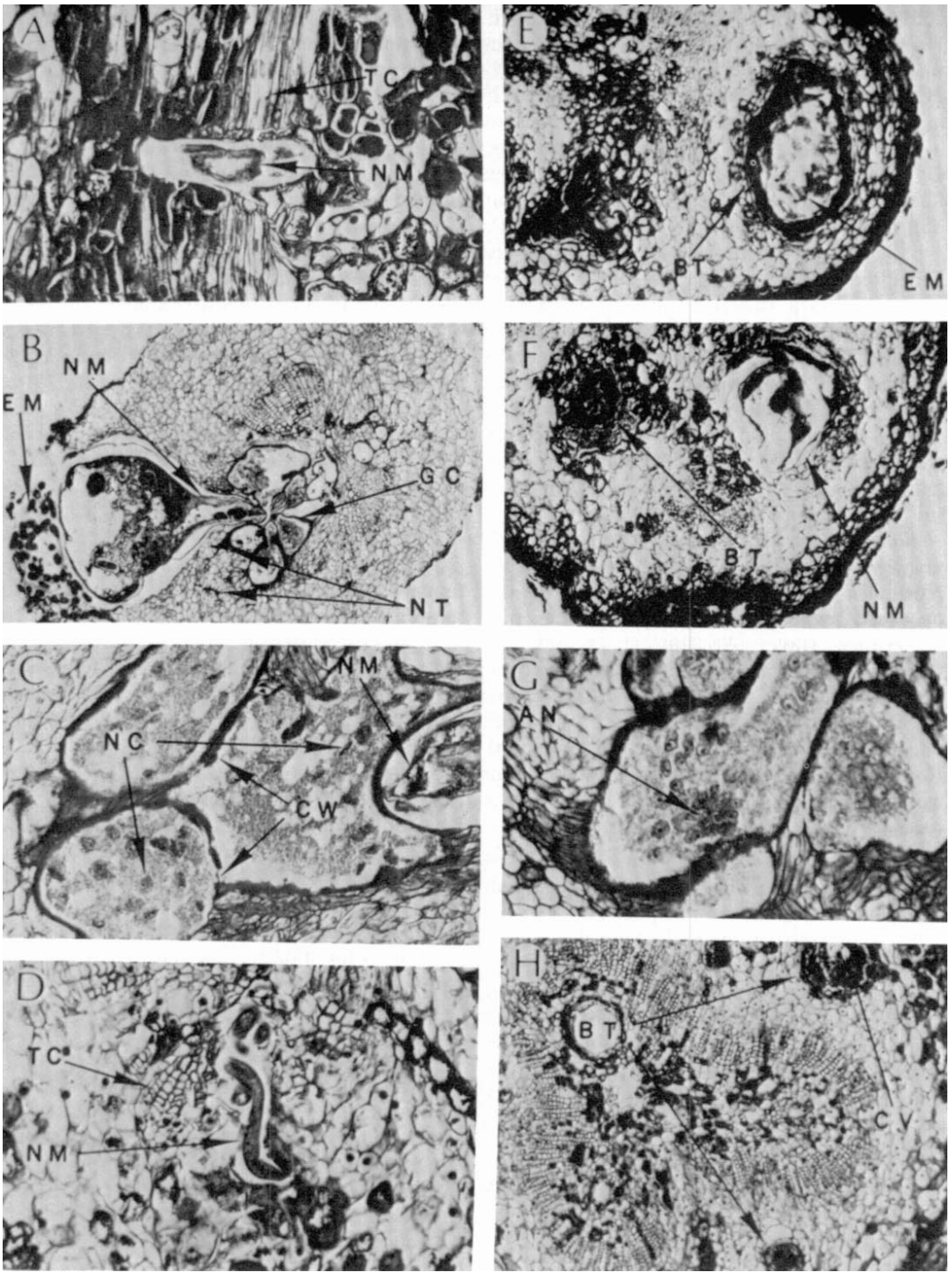
Fortune paulownia: M. incognita stunted the early growth of this species, but did not significantly reduce seedling emergence and survival when inoculated in the pre-emergence stage. When inoculated in the cotyledon stage with 10,000 larvae/pot, height and dry top weight were significantly ($P = 0.01$) decreased; when inoculated in the two-leaf stage, only dry top weight of seedlings were reduced ($P = 0.05$) 6 months after inoculation (Table 2).

Reproduction was variable between treatment replications. Larval counts/root system ranged from 0-2,736; 0-588; 0-920; and 0-357 in pre-emergence, cotyledon, two-leaf and multipetiole stages, respectively. No 1-year-old seedlings were inoculated, and no histopathological studies were conducted on this species.

Scotch pine: No significant effects of *M. incognita* on seedling emergence occurred when inoculated in the pre-emergence stage, but seedling survival was significantly ($P = 0.01$) reduced by 1,000 and 10,000 larvae/pot and significantly ($P = 0.05$) reduced by 100 larvae/pot 40 days after inoculation (Table 1). Damping-off was common in the pre-emergence stage (Fig. 1-E). *F. oxysporum* and *F. moniliforme* (Sheldon) Snyd. and Hans. were consistently isolated from diseased seedlings. The only significant ($P = 0.01$) decrease in height of scotch pine seedlings occurred when inoculated with 1,000 larvae/pot in the cotyledon stage. There was no significant difference at any other inoculum level when inoculated in later growth stages (Table 2). The number of larvae recovered ranged from 0-20; 0-132; and 0-147 larvae per root system when inoculated in pre-emergence, cotyledon and two-eight needle stages, respectively. No larvae were recovered from seedlings inoculated in the multipetiole stage. Dissection of root galls on 1-year-old seedlings 20 weeks after inoculation yielded 0-20 nematodes/gall of which 5.4% were males.

—1) Histopathology in Scotch pine—Nematode development was restricted in most cases by layers of densely red-stained

FIG. 2-(A to H). Histological reaction of forest trees to *Meloidogyne incognita* infection. **A.** Longitudinal root section of scotch pine 8 months after inoculation shows body of a nematode (NM) in the stele causing an interruption of tracheids (TC). **B.** Transverse section of black locust showing an adult female nematode (NM) with giant cells (GC) around its head and some necrotic tissues (NT). Egg mass (EM) is shown deposited on the root surface through a rupture in the cortex. **C.** Enlargement of giant cells from B showing unevenly thickened cell walls (CW), irregularly shaped nuclei (NC) and nematode (NM). **D.** A coiled mature male nematode (NM) in jack pine not associated with suberized tissue located between the tracheids (TC) and cortex. **E.** Egg mass (EM) in jack pine surrounded by several layers of suberized tissue (BT) in the cortex. **F.** A mature female nematode (NM) with minimum suberized tissue adjacent to another infection site with maximum suberized tissue (BT). **G.** An enlargement of albizzia giant cells showing pyriform agglomerated nuclei (AN). **H.** Two suberized tissue sites (BT) caused by nematodes in the cortex and a cavity assumed to be formerly occupied by a nematode in a transverse root section of *M. incognita* infected Norway spruce 20 weeks after inoculation.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cells (Fig. 1-F) indicating moderate resistance. However, some well-developed nematodes were present that were not restricted (Fig. 1-H). Fig. 1-G shows a coiled mature male nematode isolated by one or more layers of densely red-stained cells in the cortex. Hyperplasia and hypertrophy were common phenomena in the cortical and vascular parenchyma cells. In Fig. 1-H, a gravid female is shown without densely red-stained suberized cells or giant cells, but within the same section a suberized area is associated with another nematode. The absence of giant cells in association with the mature female was verified by observing 30 serial sections through an infection site. Egg masses and emerging larvae also were observed associated with other females which developed without giant cells. The nematode in Fig. 2-A ruptured the plant tracheids during its development.

Black locust: M. incognita stunted the early growth of black locust but no significant reduction in number of emerging seedlings occurred when inoculated in the pre-emergence stage. At this stage, seedling survival was significantly reduced ($P = 0.01$) only by 10,000 larvae/pot (Table 1). No significant effect in height and dry top weight occurred when other stages were inoculated. Reproduction was comparatively high on this host with as many as 91,485 larvae recovered/root system.

—1) Histopathology in black locust—Adult nematodes were well-developed. Densely red-stained and apparently necrotic cells were present around their heads and around egg masses. Hyperplastic parenchyma cells around the giant cells extended from the cortex into the stele causing a disruption of some vascular tissue. Fig. 2-B shows an adult female with its head adjacent to a cluster of giant cells in the stele region. Eggs were deposited on the root surface through a rupture in the cortex.

Irregularly shaped multinucleate giant cells had unevenly thickened cell walls (Fig. 2-C). The nuclei of the giant cells with hypertrophied nucleolei were round or elongate to spindle-shaped, and some were flattened or dish-shaped. The nuclear membrane of each large, round nucleus was indistinct, while the elongate or spindle-shaped nucleus possessed a definite membrane. The cytoplasm of the giant cell was abnormally dense and granular.

Japanese-fir: Seedling emergence was significantly reduced by 100 and 10,000 larvae/pot ($P = 0.01$) and by 1,000 larvae/pot ($P = 0.05$) when inoculated in the pre-emergence stage. This species was not included in any other experiments.

Virginia pine: Seedling survival was significantly ($P = 0.01$) reduced by 1,000 and 10,000 larvae/pot (Table 1) when pre-emergence seedlings were inoculated, but emergence was not affected. This species was not tested in older seedling stages.

—1) Histopathology in Virginia pine—Larvae were encapsulated by layers of densely red-stained cells soon after penetration and no further growth and maturation of the nematodes occurred. This species is considered to be highly resistant.

Jack pine: Seedling survival was significantly ($P = 0.01$) reduced with 100, 1,000 and 10,000 larvae/pot (Table 1) but no significant effect on seedling emergence occurred when inoculated in the pre-emergence stage. Reduction in height and dry top weight of seedlings was not significant at any inoculum level in 1-year-old seedlings. The number of nematodes per gall ranged from 0-28 of which 11.8% were males. This species was not tested in other stages.

—1) Histopathology in jack pine—The resistant reaction of this host was similar to that in scotch pine. Most nematodes that penetrated the root cortex were isolated by layers of densely red-stained cells that appeared to be suberized. No giant cells were found, but there were occasional mature females and males. Egg masses and larvae were found embedded in the root tissue and larvae apparently had no chance of escaping from the surrounding layers of heavily suberized cells (Fig. 2-E). Fig. 2-F shows a mature female with minimum evidence of a resistant host response while nearby is another infection site with a maximum resistant reaction. Hyperplasia and hypertrophy of cortical and vascular parenchyma cells were common in the infected tissue, however, there was no reduction in plant growth.

Albizzia: M. incognita stunted the early growth of albizzia but had no significant effect on seedling emergence and survival when inoculated in the pre-emergence stage. Height and dry top weight of seedlings were not significantly influenced when inoculated in the three later stages.

M. incognita completed its life cycle when inoculated in all of the plant growth stages. Good reproduction of the nematode was evident from the high numbers of larvae recovered from the roots (up to 43,960 larvae/root system).

—1) Histopathology.—Nematode-induced hyperplasia and hypertrophy of the cortical and vascular parenchyma cells was apparent, but there was no evidence of necrosis. Most nuclei of giant cells were spherical; however, some were agglomerated and pyriform with necks projecting towards the center of the group (Fig. 2-G).

Taiwania: This species appeared to be immune to *M. incognita*. There was no evidence of infection or plant injury.

Northern white cedar, red pine, Norway spruce, and white pine: These species were inoculated only in the 1-year-old seedling stage. No significant reduction occurred in height and dry top weight of seedlings at any inoculum level except northern white cedar which showed a significant ($P = 0.05$) decrease in height at 10,000 and 20,000 larvae/pot. The nematodes per gall ranged from 0-3; 0-4; and 0-5; in northern white cedar, Norway spruce, and red pine, respectively. The percentage of males was 4.5% in red pine. No nematodes were recovered from white pine.

—1) Histopathology in the Norway spruce.—Root gall sections indicated that Norway spruce was highly resistant to *M. incognita*. After penetration the larvae were encapsulated by layers of densely red-stained cells and further growth and maturation of the nematode was prevented. In Fig. 2-H, two similar infection sites are shown with highly developed layers of densely red-stained cells surrounding the area formerly occupied by larvae.

DISCUSSION

Our data show that *M. incognita* severely damages China-fir, yellow cypress, Japanese-fir, jack pine, and Virginia pine in the pre-emergence stage at certain inoculum levels. Accompanied by secondary invaders, this nematode can significantly affect the survival of black locust, China-fir, scotch pine and Virginia pine in the cotyledon stage. In the pre-emergence and cotyledon stages, *M. incognita* stunted the early growth of black locust, albizzia, and fortune paulownia seedlings; however, the seedlings recovered so that the initial stunting did not show in the final results. After the two-leaf or two-eight needle stage, black locust, fortune paulownia, yellow cypress, scotch pine, and even the highly susceptible China-fir became increasingly resistant. Growth stimulation induced by the nematode occurred on fortune paulownia and yellow cypress seedlings in the multipetiole stage at the lowest inoculum level. There was no significant influence of the nematode on the growth of scotch, jack, red and white pines and Norway spruce in 1-year-old seedlings, and growth reduction of northern white cedar occurred only at higher inoculum levels.

M. incognita increased the severity of damping-off disease which influenced the survival of black locust, China-fir, scotch and Virginia pines, and thus indicates that *M. incognita* is important to seedling stands in forest nurseries. The consistent isolation of *Fusarium* spp. from diseased seedlings suggests that these fungi were the major cause of damping-off. Severe damage to resistant conifers in the young seedling stage agrees with the results of those investigators (1, 9, 10) who consider that resistance is due to internal factors that do not prevent penetration by the larvae. Therefore, even though a nutritional relationship is not established, injury due to invasion occurs on the unsuitable host in the seedling stage.

Nematode reproduction occurred on six of the 14 tree species tested. These are, in decreasing order of ability to support reproduction, black locust, albizzia, China-fir, fortune paulownia, scotch pine, and jack pine. The above ranking, based only on recovery of larvae, may be somewhat inaccurate because in some hosts (scotch and jack pines) more larvae or egg masses appeared to be trapped within the roots than in others.

The histopathology of seven hardwood and conifer tree species showed that hyperplasia and hypertrophy were common in the cortical and vascular parenchyma cells. In jack, scotch, and Virginia pines, and in Norway spruce, heavily suberized cells which restricted the development of the nematode after penetration was the predominant form of host response.

There are many different critera to measure the immune, resistant, susceptible, tolerant, and intolerant responses of plants to root-

knot nematodes. According to Dropkin and Nelson's (3) summarization of the variation in host-parasite reaction, and in light of our histopathological studies, albizzia would be listed as a tolerant plant because the nematode completes its life cycle in host tissue and no necrosis develops. Black locust is considered as a partially tolerant plant because the nematode completes its life cycle and necrosis does not affect the plant's growth. China-fir is intolerant because, although the nematode could complete its life cycle, it was reduced in size and produced fewer eggs and larvae, but more males. Scotch and jack pines are resistant because most of the nematodes were restricted by layers of suberized cells and few completed their life cycle. Also, there was no apparent effect on host growth. Norway spruce and Virginia pine are highly resistant because no nematodes were found. However, areas of suberized cells presumed to be caused by them were observed, but there were no measurable effects on host growth. Taiwania is apparently immune to infection because there was no gall formation or effect on the plant growth. Further study is needed to determine whether or not larvae enter the roots.

Adverse conditions, such as an unsuitable or resistant host or crowding of larvae which induces nutritional deficiencies, cause an increase in the precentage of males (2, 4, 8). The increase in males in China-fir, scotch and jack pines indicates that they were unsuitable hosts for *M. incognita.*

The isolation of nematodes by suberized tissues in the roots of scotch, jack and Virginia pines and Norway spruce suggests that structural changes in the cells adjacent to the infection site acts as a physical barrier to larvae seeking a more compatible site for feeding and development. The occurrence of adult females with minimum suberized tissues in jack pine and scotch pine adjacent to a site showing a typical resistance reaction suggests that resistance is highly localized and variable within an individual host.

The absence of giant cells associated with development of adult males in China-fir and with females and males in scotch and jack pines raises a question as to whether these cells are vital to full development. Several investigators (5, 6, 8) have indicated that root-knot nematode feeds in the intercellular spaces or on many kinds of nonvacuolated thin-walled cells prior to giant cell formation. Our observations on scotch pine suggests that, in some plants, this type of feeding may be sufficient for complete development (Fig. 1-H). However, because of the relatively few adult females observed in this study, this phenomenon probably constitutes a rare exception to the notion that giant cells are vital to root-knot nematode development.

We have shown that *M. incognita* invades and causes a wide range of damage to several forest trees when inoculated in the seedling stage. The nematode may also enhance damage by other soil-borne pathogens to cause additional loss of stands in forest tree nurseries. Since trees become increasingly resistant with age, it is probable that root-knot nematodes are more of a problem in nurseries than in established forests. Because of this, forest nurserymen, as well as forest tree breeders, must take into consideration methods of limiting nematode populations by rotation, cultural practices, or chemical control.

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